

SARS-CoV-2 IgM ELISA Kit

(Catalog # E4902-100, 100 assays; Store at -20°C)

I. Introduction:

Coronaviruses are large, lipid-enveloped, single-stranded RNA viruses known to infect a wide array of hosts, including humans, bats, mice and other mammals. Amongst the seven known human coronaviruses, MERS-CoV, SARS-CoV-1 and SARS-CoV-2 are the most pathogenic, causing severe, potentially fatal respiratory and cardiovascular symptoms. The emergence of the novel SARS-CoV-2 virus, the cause of the ongoing Coronavirus Disease 2019 (COVID-19) pandemic, has proven to be the greatest public health crisis of the 21st Century. SARS-CoV-2 has a ~30 kb genome that encodes 29 proteins, including 4 structural proteins used in virion assembly, namely the Envelope (E), Membrane (M), Nucleocapsid (N) and Spike (S) proteins. The transmembrane Spike protein is a glycoprotein responsible for host-cell binding and viral entry. It is a heterotrimeric complex with two unique subunits. The S1 subunit contains the Receptor Binding Domain, which interacts with Angiotensin-Converting Enzyme 2 as a host-cell receptor, whereas the S2 subunit mediates viral membrane fusion. The S protein is highly immunogenic, with seroconversion in infected individuals typically detected between 5-21 days following the onset of symptoms. **BioVision's SARS-CoV-2 IgM ELISA Kit** is an enzyme-linked immunosorbent assay intended for identifying the early immune response to SARS-CoV-2 infection. The assay uses the spike protein S1 subunit as bait to capture anti-SARS-CoV-2 antibodies in diluted plasma or serum samples. Subsequent reaction with an enzyme-labeled anti-human-IgM conjugate and a colorimetric substrate generates color, which is proportional to the amount of anti-S1 antibodies. The presence of anti-SARS-CoV-2 S1 IgM antibodies is determined by comparing the OD_{450 nm} reading of the sample to the OD_{450 nm} reading of the negative control serum. A positive reaction suggests recent infection, as IgM antibodies are the first to appear following exposure and are generally detectable a few days after infection. Positive control serum containing a recombinant human monoclonal anti-S1 IgM antibody is included in the kit for validation. The assay is a quick, easy and accurate method for determination of recent SARS-CoV-2 infection that does not require collection or handling of infectious virus. This kit is a valuable tool to study COVID-19 epidemiology, prevalence and morbidity.

II. Applications:

- Determination of recent/prior SARS-CoV-2 infection
- Screening of SARS-CoV-2 infection rate in a given population
- Study of epidemiology, prevalence and morbidity of COVID-19

III. Sample Type:

- Human Serum or Plasma (Heparin or EDTA)

IV. Kit Contents:

Components	E4902-100	Cap Code	Part Number
Spike Protein-Coated ELISA Plate	8 x 12 Strips	--	E4902-100-1
Wash Buffer (10X)	50 ml	WM	E4902-100-2
Sample Diluent	50 ml	NM	E4902-100-3
Anti-IgM-HRP Conjugate (500X)	50 µl	Blue	E4902-100-4
Positive Control Serum	30 µl	Violet	E4902-100-5
Negative Control Serum	60 µl	White	E4902-100-6
TMB Substrate	10 ml	Amber	E4902-100-7
Stop Solution	10 ml	NM	E4902-100-8
Plate Sealing Film	2	--	E4902-100-9

V. User Supplied Reagents and Equipment:

- Multiwell microplate spectrophotometer (capable of reading absorbance at 450 nm)
- Precision multi-channel pipette and reagent reservoir

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20°C protected from light. Briefly centrifuge all small vials at low speed prior to opening. Read the entire protocol before performing the experiment. Dilute concentrated reagent stock solutions to working concentration as needed immediately prior to use.

- **Spike Protein-Coated ELISA Plate:** Do not open until ready to use. Bring to room temperature (RT) prior to opening. **After opening, immediately store remaining unused antibody-coated strips at 4°C in foil bag with desiccant** to protect strip wells from moisture.
- **Wash Buffer (10X):** Warm to RT prior to use (if crystals are present, mix gently until the crystals are completely dissolved). Prepare 100 ml of 1X Wash Buffer by diluting 10 ml of Wash Buffer (10X) with 90 ml deionized water. The 1X Wash Buffer is stable at 4°C for one month.
- **Anti-IgM-HRP Conjugate (500X):** Divide the stock into aliquots if desired and store at -20°C, protected from light. Avoid repeated freeze/thaw cycles.
- **Positive Control Serum and Negative Control Serum:** Provided as a 100-fold concentrated solution. Divide into aliquots and store at -20°C. Concentrated solution is stable for 4 freeze/thaw cycles.
- **TMB Substrate, Sample Diluent and Stop Solution:** Provided as a ready-to-use solution. Bring to RT prior to use. When not in use, reseal bottle immediately and store at 4°C, protected from light.

VII. SARS-CoV-2 IgM ELISA Assay Protocol:

1. Sample Preparation:

Note: Use proper personal protection equipment when handling biological samples.

- Collect serum or plasma samples by standard methods. We recommend using either "off-the-clot" serum (collected in tubes that are free of anticoagulants) or plasma collected with EDTA or lithium/sodium heparin. Samples exhibiting lipemia or excessive turbidity

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should be clarified using a non-binding 0.2 µm syringe filter (e.g. BioVision Cat# M4332) in order to eliminate lipid globules and other debris. Once collected, serum/plasma samples may be stored at -20°C for up to one month prior to assay.

Note: Do not use serum/plasma that is hemolyzed or contaminated with red blood cells, as this may interfere with the assay.

- b. In clean microfuge tubes, dilute serum/plasma test samples at 1:100 ratio with Sample Diluent (for example, mix 5 µl of serum or plasma with 495 µl of Sample Diluent).

2. Assay Plate Layout and Reagent Preparation:

- a. Format the microplate for the number of serum/plasma test samples to be assayed by placing a sufficient number of wells in the plate frame, including two duplicate wells for the Negative Control and one well for the Positive Control.
- b. In clean microfuge tubes, dilute both the Positive Control Serum and the Negative Control Serum at 1:100 ratio with Sample Diluent (for example, mix 2 µl of each with 198 µl of Sample Diluent, respectively).
- c. Add 100 µl of diluted (1:100) test samples, diluted Negative Control Serum (1:100) and diluted Positive Control Serum (1:100) to the designated wells. Mix wells and cover with plate sealing film. Incubate plate for 1 hour at 25°C with gentle orbital shaking.

3. Secondary Antibody (Anti-Human IgM) Reaction:

- a. Remove the plate sealing film. Aspirate the solution from each well add 300 µl of 1X Wash Buffer to each well. Aspirate the Wash Buffer from each well and pat it dry against clean absorbent paper. Repeat this wash step a total of 4 times.
Note: A microplate washer can be used in this step and other wash steps.
- b. Prepare a working solution of 1X Anti-IgM-HRP Conjugate by diluting the 500X stock solution in 1X Wash Buffer (mix 10 µl of 500X Anti-IgM-HRP Conjugate with 4990 µl of 1X Wash Buffer). Prepare sufficient amount of 1X Anti-IgM-HRP to add 100 µl per well.
Note: Make the 1X Anti-IgM-HRP Conjugate solution fresh. Do not store or reuse the diluted Anti-IgM-HRP Conjugate.
- c. Add 100 µl of 1X Anti-IgM-HRP Conjugate to each well. Mix wells and cover with plate sealing film. Incubate plate for 30 min at 25°C with gentle orbital shaking.
- d. Aspirate the solution from each well and add 300 µl of 1X Wash Buffer to each well. Aspirate the Wash Buffer from each well and pat it dry against clean absorbent paper. Repeat this wash step a total of 4 times.

4. **Measurement:** Remove any residual Wash Buffer from the wells and add 100 µl TMB Substrate to all wells. Incubate the plate (protected from light) for 10 min at 25°C. Following the 10 min incubation period, add 100 µl Stop Solution into each well. Measure the absorbance at 450 nm in a microplate reader within 15 min of adding Stop Solution.

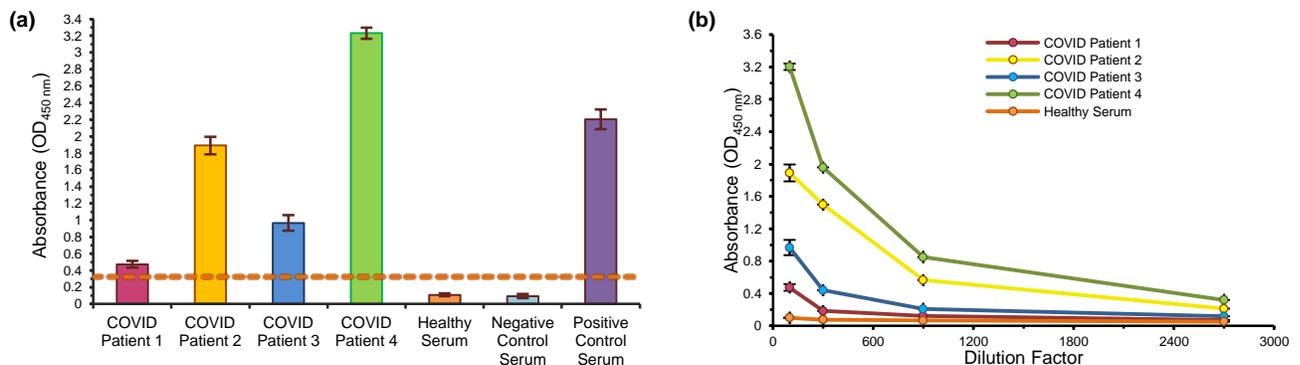
Note: The 10 min TMB reaction time must be consistent for all wells. To minimize variation in substrate incubation time, we recommend using a multichannel pipette and reagent reservoir to add TMB Substrate and Stop Solution.

5. **Calculation:** Calculate the mean absorbance ($OD_{450\text{ nm}}$) value obtained for the Negative Control Serum (A_{NC}) and all of the Test Samples (A_{Sample}). The cutoff for a presumptive positive sample (at a 1:100 dilution factor) is:

$$A_{\text{Sample}} \geq 1.1 \times (A_{NC} + 0.2)$$

Note: When diluted at 1:100, the Negative Control Serum must generate an $OD_{450\text{ nm}}$ signal less than 0.2 for the test to be valid. The Positive Control Serum should generate an $OD_{450\text{ nm}}$ greater than 1.0 when diluted at 1:100 ratio.

If desired, positive samples may be run at further dilutions to determine the antibody titer or to calculate the area under the curve (AUC) of sample dilution curves. **Negative results do not preclude acute SARS-CoV-2 infection.** The sensitivity of IgM detection early after infection is unknown. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.



Figures: (a) Detection of Anti-SARS-CoV-2 IgM antibodies in serum samples (dilution 1:100). COVID-19 patient serum samples were obtained from individuals that had a positive test for SARS-CoV-2 viral RNA by RT-PCR from nasopharyngeal swab specimen. All patient serum samples were collected at 5-20 days following PCR confirmation of SARS-CoV-2 infection. Healthy serum was donor-pooled off-the-clot serum obtained from healthy individuals prior to the COVID-19 pandemic. The response from Negative Control Serum was used to determine the cutoff for positivity (indicated by the dashed line). (b) Serial Dilutions of COVID-19 patient sera versus healthy serum. Calculated AUC values for COVID-19 patient samples were 1.96-, 9.98-, 3.69- and 14.43-fold greater than that of pre-pandemic healthy human serum, respectively. Data presented are mean ± SD of triplicate or greater wells.

VII. Related Products:

ACE2 Inhibitor Screening Kit (K310)
SARS-CoV-2 IgG ELISA Kit (E4901)
Coronavirus IgM/IgG Antibody Detection Card (K1463)
Recombinant SARS-CoV-2 Nucleoprotein (P1523)

ACE2 Activity Assay Kit (K897)
Coronavirus Rapid RT-qPCR Detection Kit (K1461)
Recombinant SARS-CoV-2 3C-like Protease (P1550)
Human CellExp™ SARS-CoV-2 Spike Protein (P1524)

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